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# 濒危植物杜鹃红山茶的细胞学研究*

王 霜[1,2], 王仲朗[1**], 梁 静[1], 管开云[1]
(1 中国科学院昆明植物研究所, 云南 昆明 650204; 2 中国科学院研究生院, 北京 100049)

**摘要**: 报道了杜鹃红山茶 (*Camellia azalea*) 的细胞学资料。其间期核为球形前染色体型, 有丝分裂前期染色体为中间型。核型公式为 2n = 2x = 30 = 28m (1SAT) + 2sm, 核型类型属于 Stebbins 的 1B 型。第 3、4、9、10、11、12 条染色体的长臂上具有次缢痕, 第 15 条染色体的短臂上具随体。细胞学证据支持杜鹃红山茶在山茶组中较为原始的推断, 并为人工杂交育种时亲本的选择提出了一些建议。

**关键词**: 杜鹃红山茶; 核型; 育种
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# Karyological Study on the Endangered Species *Camellia azalea* (Theaceae)*

WANG Shuang[1,2], WANG Zhong-Lang[1**], LIANG Jing[1], GUAN Kai-Yun[1]
(1 *Kunming Institute of Botany, Chinese Academy of Sciences*, Kunming 650204, China;
2 *Graduate University of Chinese Academy of Sciences*, Beijing 100049, China)

**Abstract:** The chromosome number and karyotype of *Camellia azalea* were reported here for the first time. The species showed in somatic cells the round prochromosome type of the interphase nuclei and the interstitial type of the prophase chromosomes. It was a diploid, and the karyotype could be formulated as 2n = 2x = 30 = 28m (1SAT) + 2sm, belonging to Stebbins' 1B type. The third, fourth, ninth, tenth, eleventh and twelfth chromosomes had secondary constriction respectively, and one satellite had been found on the fifteenth chromosome. The cytological results supported the suggestion that *C. azalea* was primitive species in Sect. *Camellia* of the genus *Camellia*. A clue on selecting parents was also proposed due to the chromosome numbers and the position in Sect. *Camellia*.

**Key words:** *Camellia azalea*; Karyotype; Breeding

*Camellia azalea* C. F. Wei was firstly discovered and published by Wei (1986). The next year, Ye (1987) published this species under the name of *Camellia changii* and now as a synonym. It is naturally distributed in E'huangzhang Natural Reserved Area of Yangchun County in Guangdong Province, China. It is an endangered species and has a very limited geographic distribution, now only about 1000 individuals in the wild and has been classified as one of first class protected plants in China. Luo *et al*. (2005) investigated the genetic diversity of three endemic and endangered species of the family Theaceae in Guangdong, China and showed that *C. azalea* had very low genetic diversity. Studies of propagation and breeding on this species have achieved progress in China in recent years (Zhang and Liang, 2003; Li *et al*. 2004). In 2006, two new cultivars have been developed and registered from the cultivated plants by Fu (2006) and Li (2006). The chromosome number and karyotype of *C. azalea* were firstly studied in this paper, so as to

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** Author for correspondence. E-mail: wang@mail.kib.ac.cn; Tel.: 0871-5223702; Fax.: 0871-5150227
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**作者简介**: 王霜 (1982-) 女, 在读硕士研究生, 主要从事山茶的育种与繁殖研究。

give some cytological evidences on its studies of taxonomy, and to reveal genetic background of chromosomes on cross-breeding program .

## Materials and methods

Materials Cuttings of *C. azalea* were collected from Yunfeng Camellia Garden in Zhejiang in August 2005, then propagated in Kunming Botanical Garden . The growing root tips were used for chromosome research . The voucher specimen (Wang Zhonglang 05 - 081) was collected from the same plant which cuttings were taken, and were deposited in the Herbarium of Kunming Institute of Botany (KUN) .

**Methods** The fresh root tips of 5 mm long were cut off from rooted cuttings, pretreated with 0.002 mol/L 8-hydroxyquinoline solution at room temperature for 5 h, then fixed with Carnoy's fluid (absolute alcohol∶glacial acetic acid = 3∶1) at 5℃ overnight . The fixed root tips were macerated in a mixture of 1 mol/L hydrochloric acid and 45% glacial acetic acid (1∶1) at 60℃ for 6 min, immediately rinsed with distilled water at room temperature for 3 - 5 min, then stained and squashed with Carbol Fuchsin . The cytological classification of the interphase and mitotic prophase followed Tanaka (1971; 1977) . Chromosomes of more than 20 cells were counted . The parameters of the chromosomes were based on the measurements of 5 cells . The use of symbols for the description of chromosomes followed Levan *et al* . (1964) and the classification of karyotype symmetry followed Stebbins (1971) .

## Results

At mitotic interphase (Fig . 1: B), the nuclei was characterized by some deeply stained, round prochromosomes of almost equal size with numerous minute grains distributed in the nucleus, and could be categorized as the round prochromosome type . At mitotic prophase (Fig . 1: D), heterochromatic and euchromatic segments were distinguishable but without clear boundaries between them, and the heterochromatic segments were distributed in the distal and interstitial regions as well as the proximal regions . This corresponded to the interstitial type . The above characteristics have been shown in all the observed species in the genus *Camellia* without any exception (Kondo *et al* . 1986; Li and Liang 1990; Gu and Sun 1997; Zhang and Min, 1999; Min, 2000) .

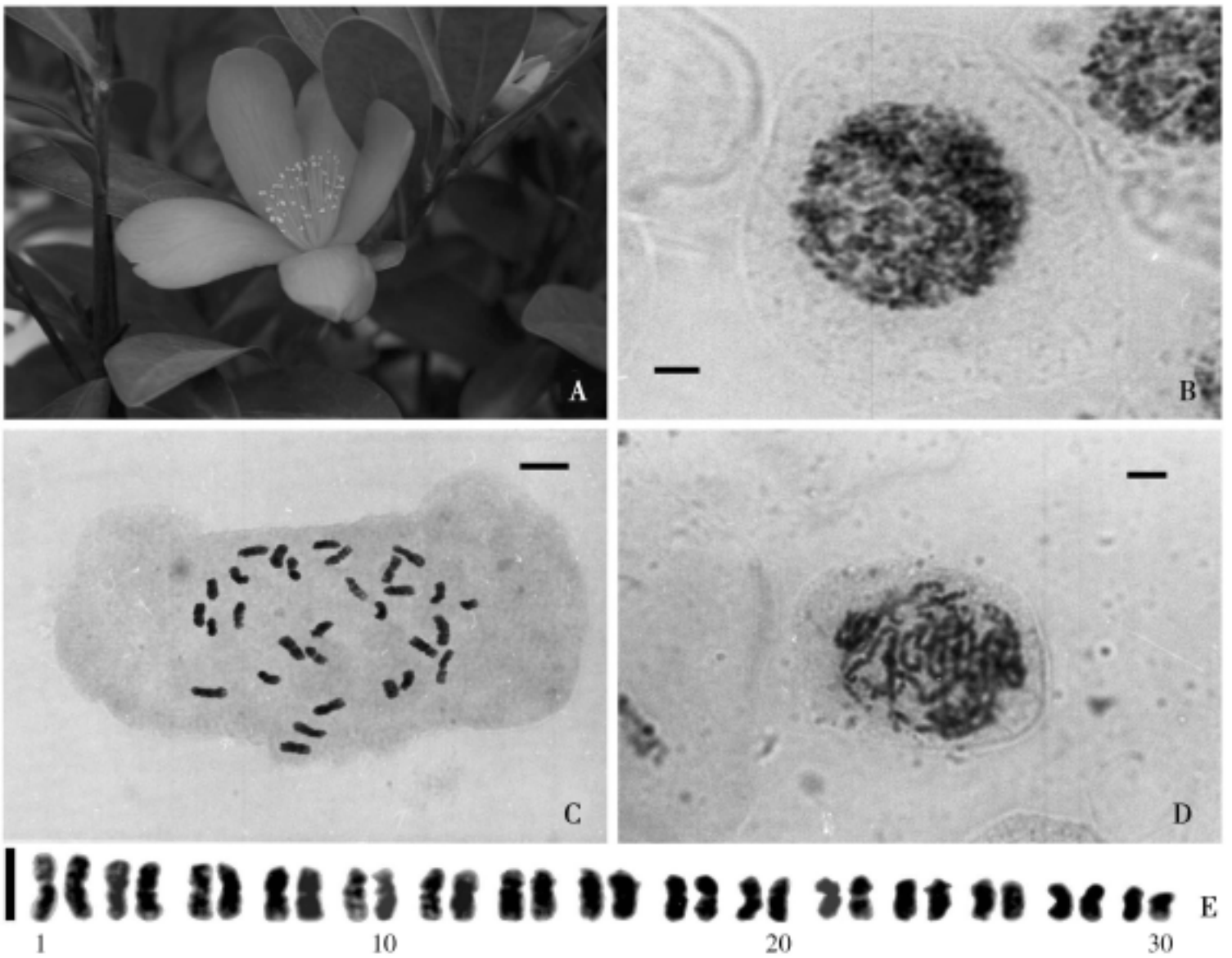


Fig . 1 The plant and cytological observation of *Camellia azalea*

Flower and leaves (A), Interphase nuclei (B), Metaphase chromosomes (C), Mitotic prophase (D), karyotype (E), Bar = 4 μm

Table 1 Karyomorphological parameters of *Camellia azalea*

| No . | Relative length | Ratio | Type | No . | Relative length | Ratio | Type |
|---|---|---|---|---|---|---|---|
| 1 | 2.74 + 2.00 = 4.74 | 1.37 | m | 16 | 1.95 + 1.45 = 3.40 | 1.34 | m |
| 2 | 2.60 + 2.06 = 4.66 | 1.26 | m | 17 | 1.87 + 1.37 = 3.24 | 1.36 | m |
| 3 | 2.09 + 2.06 = 4.15 | 1.01 | $m^{SC}$ | 18 | 1.77 + 1.38 = 3.15 | 1.28 | m |
| 4 | 2.11 + 1.99 = 4.10 | 1.06 | $m^{SC}$ | 19 | 1.52 + 1.49 = 3.01 | 1.02 | m |
| 5 | 2.10 + 1.99 = 4.09 | 1.06 | m | 20 | 1.59 + 1.38 = 2.97 | 1.15 | m |
| 6 | 2.10 + 1.74 = 3.84 | 1.21 | m | 21 | 1.58 + 1.35 = 2.93 | 1.17 | m |
| 7 | 1.88 + 1.87 = 3.75 | 1.01 | m | 22 | 1.58 + 1.27 = 2.85 | 1.24 | m |
| 8 | 1.84 + 1.82 = 3.66 | 1.01 | m | 23 | 1.52 + 1.26 = 2.78 | 1.21 | m |
| 9 | 2.36 + 1.24 = 3.60 | 1.90 | $sm^{SC}$ | 24 | 1.51 + 1.27 = 2.78 | 1.19 | m |
| 10 | 2.37 + 1.22 = 3.59 | 1.94 | $sm^{SC}$ | 25 | 1.40 + 1.38 = 2.78 | 1.01 | m |
| 11 | 1.89 + 1.62 = 3.51 | 1.17 | $m^{SC}$ | 26 | 1.38 + 1.35 = 2.73 | 1.02 | m |
| 12 | 1.84 + 1.67 = 3.51 | 1.10 | $m^{SC}$ | 27 | 1.44 + 1.17 = 2.61 | 1.23 | m |
| 13 | 2.16 + 1.34 = 3.50 | 1.61 | m | 28 | 1.42 + 1.16 = 2.58 | 1.22 | m |
| 14 | 1.98 + 1.52 = 3.50 | 1.30 | m | 29 | 1.19 + 1.17 = 2.36 | 1.02 | m |
| 15 | 2.14 + 1.34 = 3.48 | 1.60 | $m^{S}$ | 30 | 1.12 + 1.01 = 2.13 | 1.11 | m |

$^{S}$: chromosome with satellite; $^{SC}$: chromosome with secondary constriction

At mitotic metaphase, the chromosome number of *C. azalea* was determined to be 2n = 30 (Fig . 1: C) . Absolute chromosome lengths ranged from 1.8 to 3.8 μm and therefore could be classified as the small chromosome type . The size of chromosomes within each complement varied continuously and was formulated as 2n = 2x = 30 = 28m (1SAT) + 2sm (Fig . 1: E; Table 1) . Six chromosomes displayed secondary constriction on the long arms of the 3rd, 4th, 9th, 10th, 11th and 12th chromosomes . The 15th chromosome had a satellite on the short arm . The relative length of chromosomes ranged from 2.13 to 4.74 . The mean arm ratio was 1.25 . The ratio of longest chromosome to shortest one was 2.23 . No chromosome had an arm ratio more than 2.0 . Thus the karyotype asymmetry was classified as type 1B .

## Discussion

### The phylogeny status of *Camellia azalea*

The genus *Camellia* is distributed in East Asia and contained a lot of important economic plants . According to the latest revision on this genus, there are about 120 species throughout the world (Min 2000) . Nearly half of total species in *Camellia* had chromosome reports already (Zhang and Min, 1999) . *C. azalea* was placed in Sect . *Camellia* in this genus (Min and Brtholomew, 2007; Gao *et al* .2005; Min 2000) . Zhang and Min (1998) had ever summarized the chromosome reports on this section, and indicated that there was a clear direction in Sect . *Camellia*, namely the karyotype was from symmetry to asymmetry, and the chromosome number was from diploid to polyploid . They also showed that there were only two kinds of karyotype, 2A and 2B, in Sect . *Camellia* in the previous studies . The chromosomes observed in this study displayed that *C. azalea* was a diploid and had relatively high symmetrical 1B karyotype, much primitive compared to those in the same section . Wu *et al* . (2003) suggested that two widely cultivated groups of *Camellia recitulata* and *Camellia japonica* were probably derived from primitive species, *C. azalea* and *Camellia semiserrata* which were distributed in the north margin of tropical zone . The cytological results of *C. azalea* supported the above suggestion .

### Breeding significance

*Camellia azalea* has a very special flowering season . Most species of the genus *Camellia* bloom from December to February, while *C. azalea* is in full bloom mostly from May to October in the wild . Some plants of *C. azalea* can even bloom all the year round under cultivation . Its flowers are bright red, nearly distinct, obovate to long obovate (Fig . 1: A) . Its leaf is very unique as well with entire margin, glabrous surfaces, obovate to obcordate-laneolate (Min and Bartholomew, 2007) . Therefore it is a very precious breeding material to breed new cultivars with long flower season and with special leaves . Control pollination is one of the most important methods in camellia breeding, but

interspecific hybridization is commonly limited by compatibility . Cytological research could give a clue on selecting parents . After more than 20-year experiments on artificial hybridization, Xia (2003) concluded that the combination between species in the same section had higher compatibility, and it was easier to cross between diploid and polyploidy, if the combination was among different sections . *C. azalea* was a diploid, and was placed in the sect . *Camellia*, so it is better to select those in the same section, or polyploid camellia if in different section, so as to improve the cross-compatibility . If *C. azalea* is selected as pollen parent, some experiments on the storage of pollens should be done first, because both parents have different flower seasons .

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